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J. Nutr. 1994;124:1891-1897

PNAS USA 1993;90:537-541

Biochem. Biophys. Acta 1994;1204:243-249

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Critical Review

Selenoprotein P. A Selenium-Rich Extracellular Glycoprotein[1]

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Division of Gastroenterology, Department of Medicine and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN 37232

ABSTRACT Selenoprotein P is a glycoprotein that has been purified from rat and human plasma. In selenium-replete rats it contains 65% of the plasma selenium and its concentration is 25–30 mg protein/L. In selenium-deficient rats its concentration is <3 mg protein/L. The plasma half life of ^{75}Se in selenoprotein P is 3 to 4 h, indicating a rapid turnover. Purified rat selenoprotein P contains 7.5 ± 1 selenium atoms per molecule as selenocysteine. The sequence of the cloned cDNA predicts 10 selenocysteine residues, which suggests that the protein in plasma is a modification of the predicted one. Deduced amino acid sequence identity between rats and humans is 72%. The 3′ untranslated region of selenoprotein P cDNA contains two predicted stem loops of the type essential for selenocysteine incorporation. Northern analysis indicates that selenoprotein P is expressed by many tissues. Hepatic selenoprotein P mRNA level, but not its transcription, decreases during selenium deficiency. The decrease is less than the decrease of glutathione peroxidase mRNA, however. Selenoprotein P is postulated to serve as an extracellular oxidant defense because its presence correlates with selenium protection of selenium-deficient rats against diquat-induced lipid peroxidation and liver necrosis. More research will be required to test this hypothesis and to establish the biochemical function of selenoprotein P. J. Nutr. 124: 1891–1897, 1994.

INDEXING KEY WORDS:

- *selenium* • *selenoproteins*
- *selenocysteine* • *oxidant defense*

Many biological effects of selenium are exerted by selenoproteins. Over the last 7 years the mechanism of selenium incorporation into bacterial selenoproteins has been elucidated (Böck 1994), and several features of the process in eukaryotes have been established. In both systems selenocysteine is synthesized from serine. Selenocysteine is then incorporated into the primary structure of the protein at a UGA codon in the open reading frame of mRNA. This complicated process requires a number of specific components including a unique tRNA, several proteins, and a complex secondary structure in the mRNA. At the time of this writing, eight selenoproteins had been identified (**Table 1**). Some were recognized when a UGA codon was found in the open reading frame of their mRNAs. Others were identified by their selenocysteine content.

Evidence for selenoprotein P was presented in the early 1970s when a plasma protein was shown to incorporate ^{75}Se rapidly (Burk 1973, Millar et al. 1972). In 1977 Herrman reported attempts to purify this protein from rat plasma. He was unsuccessful because of its lability, but he was able to show that it was distinct from glutathione peroxidase and that it bound to heparin (Herrman 1977). In the early 1980s our group reported that selenium administered to selenium-deficient rats was rapidly incorporated into selenoprotein P (called ^{75}Se-P by us) in preference to glutathione peroxidase (Burk and Gregory 1982). Tappel's group established that this protein contained selenium in the form of selenocysteine and proposed the name selenoprotein P because of its plasma location (Motsenbocker and Tappel 1982). Neither Tappel's group nor ours succeeded in efforts to purify selenoprotein P to homogeneity using conventional means.

PURIFICATION AND CHARACTERIZATION

Purification of rat selenoprotein P was finally achieved with immunoaffinity chromatography (Yang et al. 1987). Hybridomas were produced from spleen cells after inoculation of mice with a partially pure preparation of selenoprotein P. Selective screening of the hybridomas with ^{75}Se-labeled selenoprotein P was used to obtain monoclonal antibodies that could

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TABLE 1

Animal selenoproteins

Selenoprotein	Reference
glutathione peroxidases	
cellular or classical	Rotruck et al. 1973
plasma or extracellular	Takahashi et al. 1987
phospholipid hydroperoxide	Ursini et al. 1985
gastrointestinal	Chu et al. 1993
selenoprotein P	this review
type I iodothyronine 5′ deiodinase	Berry et al. 1991a
sperm mitochondrial capsule selenoprotein	Kleene 1994
selenoprotein W	Vendeland et al. 1993

precipitate native selenoprotein P. The monoclonal antibodies were attached to support material for chromatography columns. Several types of support material have been employed, and the immunoaffinity columns have been used to purify the protein from plasma in a single step or after a preliminary step (Read et al. 1990). With a 1-mL column, quantities of selenoprotein P up to 300 μg have been prepared with a yield of ~50%.

Several antibody preparations have been employed to study selenoprotein P. The monoclonal antibodies used to purify the protein bind to native selenoprotein P but fail to bind to selenoprotein P on Western blots. This suggests that they recognize epitopes on the protein that are destroyed by denaturation during electrophoresis. Polyclonal antisera were raised by inoculating rabbits with purified protein, and those antibodies recognize both native and denatured rat selenoprotein P. Monoclonal and polyclonal antibodies have been used in RIA techniques to quantify selenoprotein P (Read et al. 1990). The assays were standardized with purified selenoprotein P that had been quantified by amino acid analysis. Thus, selenoprotein P values are given in weight of its protein component.

Subjection of purified selenoprotein P to SDS-PAGE resulted in a 57-kDa protein band that also stained intensely with dansyl hydrazine-periodic acid Schiff glycoprotein stain (Read et al. 1990). Digestion of the protein with *N*-glycanase before electrophoresis led to the presence of two bands (48 and 43 kDa) with less intense carbohydrate staining. Treatment with *O*-glycanase had no additional effect. This indicates that selenoprotein P is a glycoprotein. The glycosylation seems to be N-linked and there is evidence of microheterogeneity.

Several batches of purified selenoprotein P were analyzed for selenium content and found to have 7.5 ± 1 selenium atoms per molecule based on a peptide weight of 43 kDa (Read et al. 1990). Amino acid analysis indicated that all the selenium was present in the form of selenocysteine. Purified protein was fragmented at the methionine residues by cyanogen bromide treatment, and two major peptides of approximately 20 and 40 kDa resulted (Read et al. 1990). Amino acid analysis of these peptides revealed that the 20-kDa fragment was richer in selenocysteine than the 40-kDa fragment. Those results confirmed that selenoprotein P contains many selenocysteine residues and indicated that these residues are concentrated into one or more selenium-rich regions.

MOLECULAR BIOLOGY

A partial cDNA for selenoprotein P was isolated and cloned from a rat liver expression library using a polyclonal antibody preparation. The partial cDNA was used to screen a λZAP II rat liver library and obtain a full-length cDNA (Hill et al. 1991). This rat cDNA was used for screening a human liver library, resulting in isolation and cloning of a full-length cDNA. A human heart selenoprotein P cDNA was also isolated and cloned (Hill et al. 1993). The cDNAs were sequenced, and the human heart and liver cDNAs were found to be essentially identical.

The cDNAs contain complete open reading frames that code for 385 amino acids in rats and 381 in humans. Deduced amino acid sequences for rat and human proteins are shown in **Figure 1**. The first 19 amino acids of each sequence constitute a typical signal peptide to direct secretion of the protein by the cell. N-Terminal sequencing of the proteins showed that the 20th amino acid of the deduced amino acid sequence was the first amino acid of the native protein (Åkesson et al. 1994, Read et al. 1990). This finding confirms that a signal peptide is present and is

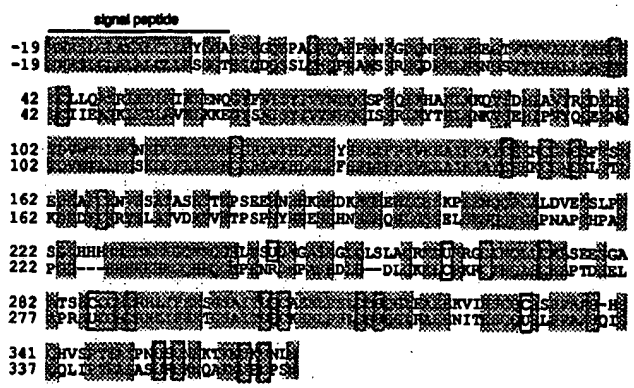

FIGURE 1 Deduced amino acid sequences of rat (upper sequence) and human (lower sequence) selenoprotein P. The signal peptide is indicated as amino acid residues -19 to -1. The mature protein begins with amino acid residue 1. Selenocysteine is designated by U. Amino acids that are identical between species are shaded. Selenocysteine and cysteine residues of the mature protein are boxed.

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consistent with the observation that selenoprotein P is an extracellular protein.

Sequence identity between rats and humans is 69% at the nucleotide level and 72% at the amino acid level (shaded in Fig. 1). The least well-conserved amino acid is asparagine at 53%. Best conserved are methionine and tryptophan, of which only two residues each are present. Peptide weights of the proteins excluding the signal peptides are 41,052 for rat and 41,229 for human. Five typical N-glycosylation sites are present in the rat sequence and six in the human (Hill et al. 1993). Two of them occupy identical positions in both species. Characterization of the bound carbohydrates has not yet been reported.

The deduced amino acid sequence of rat selenoprotein P is highly unusual. There are 10 in-frame TGA codons in the cDNA (UGAs in mRNA) that designate selenocysteine incorporation. Figure 1 shows that nine of the selenocysteine residues (represented by U) are located in the C-terminal one-third of the polypeptide. Four of them are in the last 15 amino acids. The other selenocysteine residue is the 40th amino acid in the deduced mature protein. The 20-kDa peptide isolated after cyanogen bromide cleavage was analyzed by N-terminal sequencing, and comparison of it with the deduced sequence of rat selenoprotein P indicated that it began with amino acid number 248 and thus contained the selenium-rich region of the sequence (Fig. 1).

The positions of the cysteine residues and selenocysteine residues of the protein are boxed in Figure 1. Ten cysteines are interspersed among the nine selenocysteines in the C-terminal region. Analysis of the sequence of selenoprotein P suggests that these amino acids are related functionally. Each of these amino acids is well conserved between rats and humans. However, when they are considered together, conservation is even higher (96%). This occurs because at three positions a selenocysteine residue is present in one species and cysteine residue is present in the other. Thus, either amino acid seems to be able to meet the requirements of several positions in the sequence.

Characterization of the purified protein indicated a selenium content of 7.5 ± 1 atoms per molecule, whereas 10 selenocysteine residues are predicted by the cDNA. It is not known presently why this discrepancy exists. It is possible that some selenocysteine is destroyed during the preparation for analysis. However, a modification, such as early truncation, might reduce the selenium content of selenoprotein P. With so many selenocysteines near the C terminus, early termination at one of the UGAs or post-translational cleavage of a C-terminal fragment would reduce the selenium content. Complete amino acid sequencing of the protein will be required to explain the discrepancy between the predicted selenium content and that actually measured.

Selenoprotein P has a predominance of basic amino acid residues (17.2%) over acidic ones (10.4%). The presence of basic domains is a characteristic of proteins that bind to heparin. Figure 1 shows that selenoprotein P has two heavy concentrations of histidines (amino acids 185–198 and 225–234). The more C-terminal of them includes a run of seven histidines followed by a lysine, histidine, lysine for a total of 10 consecutive basic amino acid residues in the rat protein. Such basic domains have been associated with binding of proteins to cell membranes (Hancock et al. 1990).

The 3′ untranslated regions (3′utr) of eukaryotic selenoprotein mRNAs have stem-loop structures that are necessary for decoding the open reading frame UGAs as selenocysteine (Berry et al. 1991b). Computer analysis of the 3′utr of selenoprotein P identified two such stem loops (Hill et al. 1993), as shown in **Figure 2**. Comparison of rat and human 3′utr revealed that both stem loops were highly conserved, suggesting importance. In addition, the comparison identified another conserved area that extends from the end of the open reading frame to the first stem loop. No secondary structure was predicted in this region and its function is not known.

Berry et al. (1993) studied the selenoprotein P 3′utr stem loops in their type I iodothyronine 5′ deiodinase transient expression system. Expression by COS-7 cells of the 5′ deiodinase, which contains one selenocysteine residue, requires transfection with the open reading frame and 3′utr that contains one stem loop. Berry and colleagues (1993) substituted the selenoprotein P stem loops individually and together for the native 3′utr stem loop. Substitution of the entire selenoprotein P 3′utr facilitated expression of the 5′ deiodinase at 3.7 times the activity of the native 3′utr. Substitution of the selenoprotein P 3′utr conserved region (Fig. 2, shaded box) and the first stem loop gave 2.9 times the activity of the native 3′utr. The second stem loop was also active when substituted for the native stem loop. Its activity was similar to that of the native stem loop. This indicates

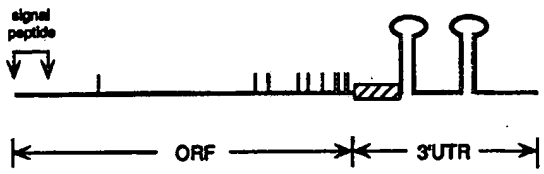

FIGURE 2 Relation of selenocysteine residues of selenoprotein P with features of its 3′utr. The open reading frame (ORF) and the 3′utr are indicated. Selenocysteine residues are indicated by the vertical lines in the ORF. The shaded box represents a region highly conserved between rats and humans. It stretches from the termination codon, which is TAA, to the first stem loop. Both stem loops are also highly conserved, but the region between them is not.

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that stem loops vary in their ability to support UGA readthrough. The first stem loop of rat selenoprotein P is the most active stem loop tested to date.

Selenoprotein P has not yet been expressed, so its need for two stem loops in the 3'utr has not been examined. Figure 2 shows the relation of the stem loops with the positions of selenocysteine in the sequence. It is possible that the first stem loop serves certain UGAs and the second serves others. Alternatively, complete synthesis might be possible using only one stem loop, with the second one serving to increase efficiency. Because the precise function of the stem loops has not been established, understanding why there are two of them in the selenoprotein P mRNA must await further experimental results.

PHYSIOLOGY

Selenoprotein P and plasma glutathione peroxidase are the only known plasma selenoproteins. The concentration of selenoprotein P in plasma and serum of selenium-replete rats is 25–30 mg/L (Read et al. 1990). An experiment was performed to determine the percentage of plasma selenium present in selenoprotein P (Read et al. 1990). Serum was passed over an immunoaffinity column made with a monoclonal antibody to selenoprotein P. This column removed >90% of the selenoprotein P and >60% of the selenium without affecting glutathione peroxidase activity. Thus, selenoprotein P contains 60–65% of plasma selenium in selenium-replete rats. The rest is presumably in glutathione peroxidase and small molecule forms (Kato et al. 1992).

The liver and heart synthesize and secrete selenoprotein P. After a cDNA probe was made, it was possible to determine whether other tissues express the protein. **Figure 3** shows a Northern analysis that demonstrates selenoprotein P expression in liver, kidney, heart, lung and testis. This widespread expression of selenoprotein P may be a clue to a site of its function. If the protein were needed only in the plasma, secretion by the liver alone should suffice. Plasma proteins have limited access to the interstitial space of most tissues, so expression of selenoprotein P by those tissues suggests that it is targeted for the interstitial space in them. The basic domains demonstrated in the sequence of selenoprotein P (Fig. 1) might facilitate its association with cell-surface and interstitial space carbohydrates. There is a recent report of selenoprotein P associating with plasma membranes (Wilson and Tappel 1993), which supports this possibility. Direct investigation of selenoprotein P binding by constituents of the interstitial space is needed.

Selenoprotein P is a major participant in selenium metabolism. Based on its plasma concentration, it contains 8% of the selenium in rats (Read et al. 1990). Its plasma half life has been estimated to be 4 h (Burk et al. 1991). This indicates that its synthesis is rapid, ~3% that of albumin. In contrast, extracellular glutathione peroxidase turns over more slowly (Burk et al. 1991).

Selenium deficiency causes a decrease in plasma selenoprotein P concentration to <10% of the concentration present in selenium-replete rats (Read et al.

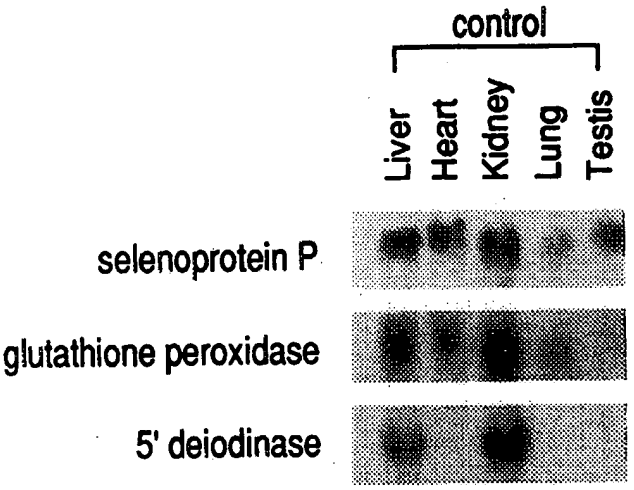

FIGURE 3 Selenoprotein P, glutathione peroxidase, and type I iodothyronine 5′ deiodinase mRNA in liver, heart, kidney, lung and testis. One-gram portions of tissues obtained from selenium-replete rats were used for isolation of total RNA as previously described (Hill et al. 1992). The tissue was homogenized in guanidine thiocyanate (1 g of tissue per 16 mL of guanidine thiocyanate reagent), layered on 5.7 mol/L CsCl, and centrifuged for 16–20 h at 150,000 × *g*. Total RNA was isolated from pelleted material. RNA samples were electrophoresed on formaldehyde (6.5%)–agarose (1%) gel and transferred to nitrocellulose with 10× SSC (1× SSC contained 0.15 mol/L sodium chloride and 0.015 mol/L sodium citrate). After drying, the membrane was prehybridized for 2 h at 42°C and hybridized overnight at 42°C with a ^{32}P-labeled cDNA probe. The cDNA probes were labeled with ^{32}P-dCTP using a nick-translation kit (cat. no. NEK-004), Du Pont NEN Products (Boston, MA). The membrane was rinsed briefly with 2× SSC and then washed for 1 h at 60°C with 1× SSC. The membrane was then exposed to Kodak XAR-5 film. The cDNA probes were the same ones used previously (Hill et al. 1992). The probe for cellular glutathione peroxidase was obtained from N. Imura (Kitasato University, Tokyo, Japan). The probe for type I iodothyronine 5′ deiodinase was obtained from J. Köhrle (University of Würzburg, Würzburg, Germany). The probe for selenoprotein P was cloned in our laboratory (Hill et al. 1991). Selenoprotein P message was detectable in all tissues examined. Cellular glutathione peroxidase message was detectable in all tissues except testis. Type I iodothyronine 5′ deiodinase message was detectable only in liver and kidney. The autoradiograph shown is representative of that obtained from three independent replicates. Tissues were obtained from rats fed a torula yeast–based diet (Burk 1987) containing 0.5 mg selenium (as sodium selenate)/kg diet for 7 wk after weaning.

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1990). Comparison of plasma selenoprotein P concentration with glutathione peroxidase activity in plasma and liver was made in rats fed graded amounts of selenium (Yang et al. 1989). As selenium was added to a selenium-deficient diet, selenoprotein P concentration rose first. Plasma and liver glutathione peroxidase activities followed. Injection of selenium-deficient rats with selenium caused a rapid rise in plasma selenoprotein P concentration to ~75% of control at 24 h (Burk et al. 1991). In the same animals, glutathione peroxidase activity in liver and plasma increased only to 6% of control. This indicates that selenoproteins are regulated differentially by selenium deficiency and that selenoprotein P synthesis has priority over glutathione peroxidase synthesis when the supply of selenium is limiting.

Levels of liver mRNA for selenoprotein P and liver glutathione peroxidase were assessed by Northern analysis in selenium-deficient rats (Hill et al. 1992). The glutathione peroxidase mRNA was 3% of control, whereas the selenoprotein P mRNA was 19% of control. This indicates that differences in mRNA underlie the differential regulation of the selenoproteins. It has been reported that the fall in glutathione peroxidase mRNA during selenium deficiency is not associated with a decrease in its transcription (Christensen and Burgener 1992, Toyoda et al. 1990). A nuclear run-on study of the transcription of selenoprotein P, cellular glutathione peroxidase, and type I iodothyronine 5′ deiodinase mRNAs was conducted in liver nuclei from control and selenium-deficient rats (**Fig. 4**). Selenium deficiency did not alter transcription rate of any of the mRNAs. Thus, the regulation of selenoprotein mRNA levels is presumably exerted through their degradation. Proteins involved in iron metabolism are regulated by binding of a protein to secondary structures in their mRNAs (Theil 1990). Selenoprotein mRNAs have secondary structures as well (Fig. 2), and these might have regulatory functions.

Tappel's group has suggested that numerous tissues possess receptors for selenoprotein P (Wilson and Tappel 1993). We evaluated this possibility by injecting ^{75}Se-labeled selenoprotein P intravenously into selenium-deficient and control rats (Burk et al. 1991). Tissues were removed from the animals after 2 h, and their ^{75}Se content was determined. Selenium status had no effect on uptake of ^{75}Se by liver, kidney or testis. However, brain in selenium-deficient rats took up several times as much ^{75}Se as brain in controls. ^{75}Se-labeled plasma glutathione peroxidase was administered to separate groups of animals as a control, and there was no difference in brain uptake of ^{75}Se between selenium-deficient and control rats. This result is compatible with a recognition site or receptor for selenoprotein P in the brain but does not provide support for the existence of such receptors elsewhere. It is possible that the affinity of selenoprotein P for cell membranes in tissues other than brain is related to its basic domains being attracted to negatively charged molecules on the cell surface and is not specific in the sense of a receptor.

FUNCTION

Tappel's group noted that administered ^{75}Se appeared in plasma selenoprotein P before it was taken up by peripheral tissues. They hypothesized that the protein serves as a selenium transport protein from the liver to peripheral tissues (Motsenbocker and

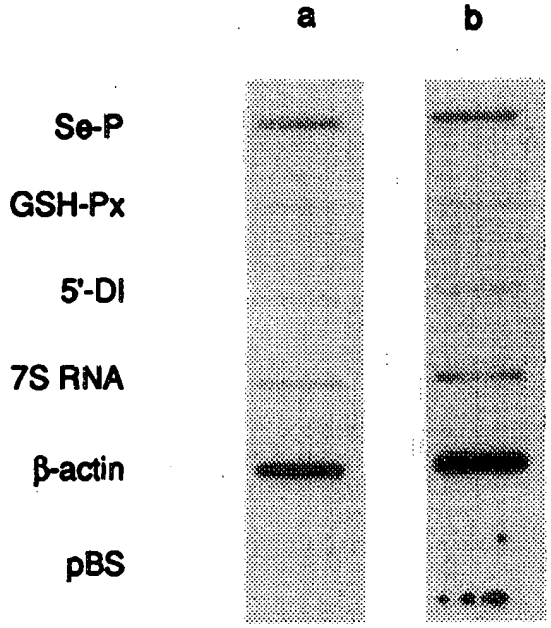

FIGURE 4 Effect of selenium deficiency on transcription of mRNAs of selenoproteins. Liver nuclei from control (lane a) and selenium-deficient (lane b) rats were studied by nuclear run-on. Weanling male Sprague-Dawley rats were fed selenium-containing or selenium-deficient diet (Burk 1987) for 3.5 mo. Glutathione peroxidase activity was measured in plasma from rats maintained on the same diet to assess the selenium deficiency produced. Glutathione peroxidase activity in plasma of selenium-deficient rats was 3% of that measured in plasma of controls [1080 ± 270 and 30 ± 3 μmol NADPH oxidized/(L plasma·min) for control and selenium-deficient rats, respectively]. Livers were obtained from three control and three selenium-deficient rats. Nuclei were isolated from each liver and stored at −80°C until ^{32}P-UTP–labeled mRNA was prepared according to the method of Schibler et al. (1983). The ^{32}P-UTP–labeled mRNA was hybridized to nitrocellulose filters that had been blotted with cDNAs for selenoprotein P (Se-P), cellular glutathione peroxidase (GSH-Px), type I iodothreonine 5′ deiodinase (5′-DI), 7S RNA (obtained from A. Diamond, University of Chicago, Chicago, IL), β-actin and Bluescript plasmid (pBS, Stratagene, La Jolla, CA). pBS was used as a negative control. Quantification and normalization of selenoprotein mRNAs to the constitutive 7S RNA and β-actin mRNA showed no effect of selenium status on transcription of these mRNAs. The autoradiograph shown is representative of nuclear run-on experiments using nuclei isolated from the six livers obtained.

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Tappel 1982). That group has also shown an association of the protein with cell membranes as noted above and has postulated that a receptor for it exists. We have also conducted studies in selenium-deficient and control rats to evaluate a transport function (Burk et al. 1991). As described above, a receptor was sought in vivo, with the only evidence for one being found in the brain. Moreover, the fact that selenium is covalently bound in the molecule and the finding that many tissues express selenoprotein P lessen the likelihood that it is a transport protein. There is recent evidence for a small molecule form of selenium in plasma that seems to be involved in its transport (Kato et al. 1992). Thus, selenoprotein P may serve to distribute selenium throughout the body, but there is little evidence that its primary function is to transport the element.

The other proposed function for selenoprotein P is as an oxidant defense. Selenium has long been associated with antioxidant properties in vivo. In combination with vitamin E deficiency in rats, selenium deficiency leads to spontaneous liver necrosis accompanied by lipid peroxidation (Hafeman and Hoekstra 1977). Treatment of selenium-deficient rats with the redox cyclers diquat and paraquat also causes liver necrosis and lipid peroxidation (Burk et al. 1980). The antioxidant effect of selenium has been generally ascribed to glutathione peroxidase (Hoekstra 1975). However, we demonstrated that injection of selenium into selenium-deficient rats protected against diquat-induced liver necrosis and lipid peroxidation before glutathione peroxidase activity had increased (Burk et al. 1980). Subsequent studies have shown that the protection is associated with the finding of significant increases in selenoprotein P (Burk et al. 1991). Thus, a temporal association between selenoprotein P and the antioxidant effect of selenium has been demonstrated. However, this does not establish directly that the protection is provided by selenoprotein P, and further work will be necessary to evaluate this possibility.

HUMAN SELENOPROTEIN P

Selenoprotein P has been purified from human plasma (Åkesson et al. 1994). The antibodies to the rat protein do not react with it, so study of the human protein has required development of separate reagents for its purification and measurement. It is a glycoprotein, and its concentration in human plasma is about a tenth of the concentration of selenoprotein P in rat plasma.

Some regions of China are low in selenium, and people living in them develop selenium deficiency. We performed a study of selenium-deficient and control Chinese populations in 1987, including measurement of plasma selenium and glutathione peroxidase activity (Xia et al. 1989). Plasma samples from that study were stored frozen and recently we analyzed them for selenoprotein P concentration. Selenoprotein P concentrations were 10 to 20% of United States population levels in the selenium-deficient group, and selenium administration to those subjects for 2 wk caused their selenoprotein P concentrations to increase (Hill et al. 1994). Thus, selenoprotein P concentrations are low in selenium-deficient humans.

FUTURE RESEARCH NEEDS

Selenoprotein P has novel features that have raised fundamental questions about selenium metabolism and function. Its study promises to advance the selenium field. Study of the molecular biology of selenoprotein P should provide insight into the mechanism of selenoprotein synthesis and, in particular, into the insertion of multiple selenocysteines. Elucidation of its structure should provide information on the relationship of cysteine to selenocysteine and indicate whether diselenides or mixed selenide-sulfides are present. Although preliminary results suggest an oxidant defense function, much more work on the function of selenoprotein P is needed. Finally, the protein needs to be studied in human diseases to determine whether it plays a role in any of them. These and related questions will stimulate research on selenoprotein P in the immediate future.

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